PRINCIPLES OF CULTIVAR DEVELOPMENT

VOLUME 1

Theory and Technique

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Macmillan Publishing Company
A Division of Macmillan, Inc.
NEW YORK

Collier Macmillan Publishers
LONDON

CHAPTER NINETEEN

ield-Plot Techniques

he fundamental purpose of plant breeding is to identify genotypes with superior rformance in commercial production. A large proportion of the time and pense devoted to cultivar development is in field evaluation of breeding ma- rial. The tests may involve genotypes in an initial stage of evaluation or those ing given final consideration for release as new cultivars. The characters valuated range from those that can be measured readily by visual examination those that must be measured with appropriate instruments. The genetic po- ntial of a genotype for some characters may be determined effectively with one or a few plants in a small plot, while for other characters extensive evaluation in larger plots may be needed.

It is the responsibility of the plant breeder to select the field-plot techniques that will provide the maximum amount of information with the resources avail- able. The challenge is to adequately test as many genotypes as possible. The resources available to plant breeders vary; usually several alternative techniques are available for character evaluation. Plant breeders must decide which tech- niques will be the most effective and efficient in their particular situation.

Detailed discussions of field-plot techniques and data analysis are provided by Gomez and Gomez (1984) and LeClerg et al. (1962). An overview of the general principles will be provided in this chapter.

SOURCES OF VARIATION

The ideal way to compare genotypes would be to grow all of them in exactly the same environment and to measure their characteristics in precisely the same manner. The differences among genotypes in this ideal situation would be due only to variation in their genetic potential; therefore, the best genotype could be chosen without error. This ideal is impossible to achieve under field conditions because of lack of uniformity in the environment to which the genotypes are

exposed. Nevertheless, the use of appropriate field-plot techniques can maxim the accuracy with which genotypes are compared and selected.

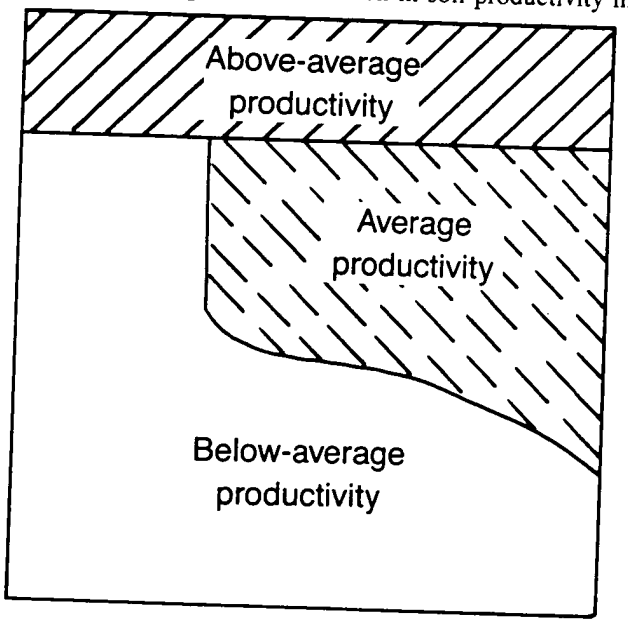
The factors that result in test conditions that are less than ideal can be refere to collectively as sources of experimental error. They include variation in t environment to which each genotype is exposed and lack of uniformity in t measurement of characters. The breeder has opportunities to minimize experi mental error by carefully selecting the site to be used for field trials, the cultura practices used in crop production, the plot size and shape, and the method o data collection.

Site Selection

Variation in the productivity of the soil is commonly referred to as soil heter ogeneity (Fig. 19-1). Causes of soil heterogeneity include variation in soil type, availability of plant nutrients, and soil moisture. The variation cannot be com pletely eliminated, but it often can be minimized by careful selection of the area in a field where plots will be grown. Soil maps are helpful for understanding the variation in soil type that is present. Soil types differ in their inherent ability to retain nutrients and moisture. Entire trials or at least an entire replication should be grown on a single soil type whenever possible.

Visual inspection of a field is important, even when a soil map is available.

Figure 19-1 Example of potential variation in soil productivity in a test area.



When a field has been identified a year in advance as a potential test site, it is useful for the breeder to look for variability in productivity of the crop grown in the area. The breeder should note variation in the terrain that may cause water to accumulate more in one place than in another. Differences in soil tillage after harvest of the previous crop may be observed that could result in nonuniformity of the area. Uneven distribution of plant or animal waste on a field should be noted as a potential contributor to variation in the availability of plant nutrients.

Before a site is chosen, information should be obtained on cultural practices that were followed in the production of previous crops, with special attention to the application of chemicals that could influence the crop that the breeder will be evaluating. The residue from herbicides applied for control of weeds in previous crops may cause damage to the crop to be tested. The following quotation from a research article by Thorne and Fehr (1970b) on soybean breeding illustrates the importance of herbicide residue:

> The strains were evaluated at Ames and Kanawha, Iowa, in 1968. . . . At Kanawha, part of the experiment was inadvertently planted in a field treated with atrazine herbicide two years before. All plots in the area were destroyed.

Previous cultural practices in a field can be especially important at research stations where crops are rotated from one field to another on a systematic basis. The research conducted on crops previously grown on a field can influence markedly the uniformity of the test site. For example, plots of oats were planted in a field at the Agronomy Research Center of Iowa State University in which soybeans had been planted the previous year. Growth of the oats varied in strips, as if nitrogen fertilizer had been applied unevenly to the field. A review of the previous soybean research revealed that the strips of oats with extra growth coincided with areas where mature soybeans had been cut and left unthreshed. The nitrogen in the soybean seeds in the strips was available to the oats the following year, and caused nonuniformity of nutrient availability in the test site.

Cultural Practices

Experimental error can be minimized by the use of uniform cultural practices for production of the crop being tested. Chemicals should be applied uniformly to the test site before, during, or after planting. Uneven soil compaction should be minimized during tillage operations. Application of supplemental water by irrigation may reduce variability in soil moisture. Weed control should be uniform; most breeders try to eliminate all weeds during the growing season to avoid experimental error caused by differential weed competition.

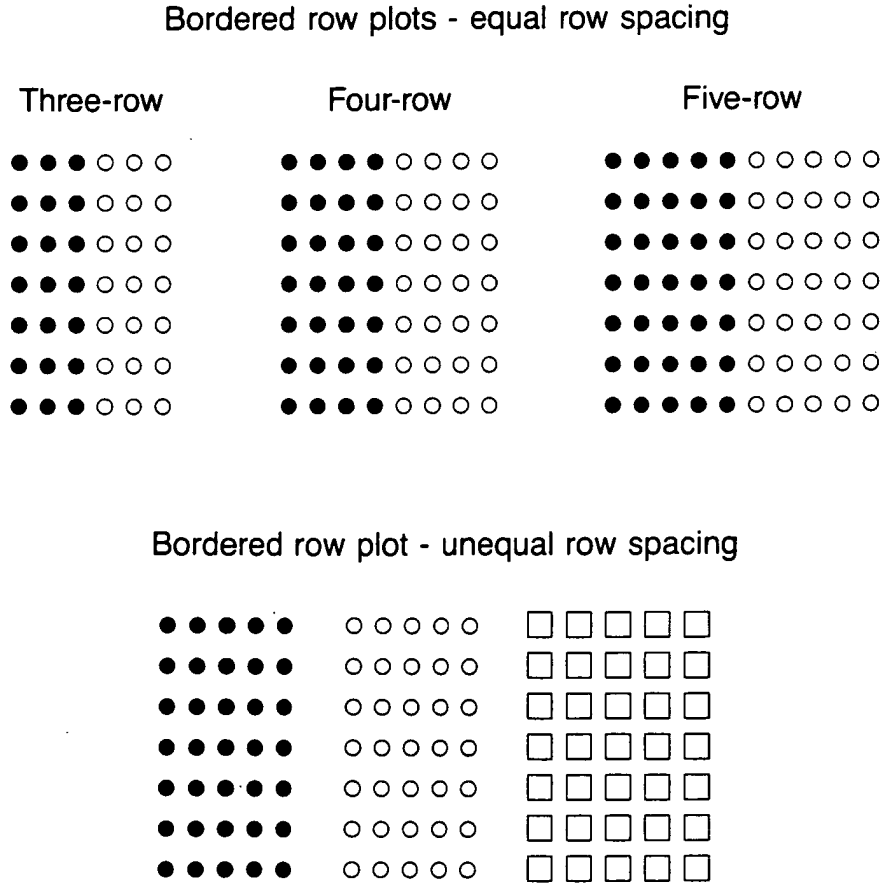
The development of equipment specifically designed for planting, managing, and harvesting research plots has permitted breeders to grow plots more efficiently. The emphasis in the design and use of any equipment must be on the uniformity with which genotypes are handled.

Plot Type

Experimental error increases whenever interplot competition causes the performance of a genotype in one plot to be altered by the performance of genotypes in adjacent plots. Interplot competition results primarily from intergenotypic competition, which is the differential ability of genotypes to compete with each other. Interplot competition is more important for the evaluation of some characters than for others. It is only through appropriate experimentation that a plot type can be identified that will provide reliable information for the character of interest.

The effects of interplot competition can be avoided by the use of plots with multiple rows in which only plants in the center rows are evaluated (Fig. 19-2). In plots with three or more rows, the outermost rows are designated as the border or guard rows. The function of the border rows is to prevent plants in adjacent

Figure 19-2 Illustration of bordered row plots with different cultivars designated as ●, ○, and □. (Courtesy of Fehr, 1978.)

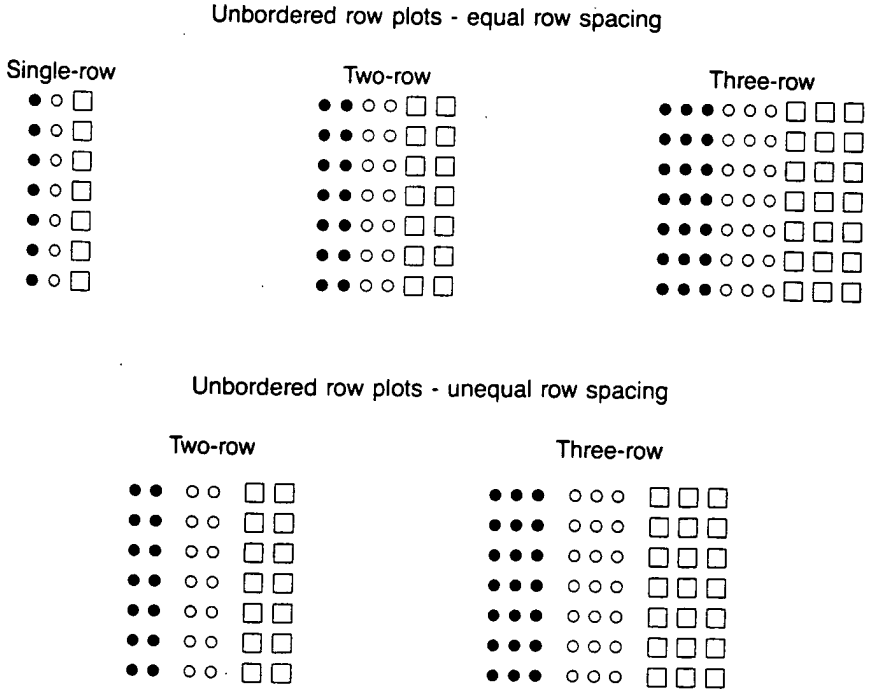


plots from influencing the performance of plants in the center of the plot. Each bordered plot can be considered a miniature field that is unaffected by neighboring fields. The spacing between plots can be greater than the within-plot spacing to facilitate the movement of equipment, particularly when narrow rows are utilized.

It would be ideal if bordered plots could be used for the evaluation of all characters that are influenced by interplot competition. That ideal is difficult to achieve when thousands of genotypes are being evaluated. Bordered plots require seed and land that do not directly provide data for a genotype. Borders take up two-thirds of the seed and land area for three-row plots and one-half for four-row plots. The cost and availability of seed and land often necessitate restriction of the use of bordered plots to the evaluation of genotypes that are being given final consideration for release as cultivars.

Interplot competition can be reduced, but not eliminated, with unbordered plots of two or more rows, all of which are used to evaluate a character (Fig. 19-3). A genotype in a single-row plot is subjected to interplot competition on both sides. Interplot competition is reduced by one-half in plots with two rows, two-thirds with three rows, three-fourths with four rows, and four-fifths with five rows. The estimated reduction of interplot competition with increasing numbers of rows is based on the fact that each row of a plot must compete on two sides. The border rows are each subjected to interplot competition on one side

Figure 19-3 Illustration of unbordered row plots with different cultivars designated as ●, ○, and □. (Courtesy of Fehr, 1978.)



but not on the other. Any rows within the two border rows are protected from interplot competition. This can be expressed as

$$\text{Reduction in interplot competition compared with single-row plot} = \frac{(\text{number of rows per plot} \times 2 \text{ sides}) - 2 \text{ sides}}{\text{number of rows per plot} \times 2 \text{ sides}}$$

$$\text{Two-row plot} = \frac{(2 \times 2) - 2}{2 \times 2} = 1/2$$

$$\text{Three-row plot} = \frac{(3 \times 2) - 2}{3 \times 2} = 2/3$$

The amount of interplot competition also can be reduced by increasing the spacing between rows of adjacent plots. Interplot competition in soybeans was evaluated with five cultivars grown in single rows spaced 100, 75, 50, and 25 cm apart (Gedge et al., 1977). The average effect of interplot competition on seed yield was 2.6 percent for the 100-cm spacing, 5.3 percent for 75 cm, 8.0 percent for 50 cm, and 17.6 percent for 25 cm.

A combination of increased row spacing between plots and a large number of rows can minimize interplot competition in unbordered plots. In the soybean example of the preceding paragraph, the average change in yield for single-row plots spaced 100 cm apart was 2.6 percent. The percentage theoretically would be reduced to 1.3 percent for two-row plots and to 0.9 percent for three-row plots. Rows within a plot are not subjected to interplot competition; therefore, the spacing between rows within a plot can be less than the spacing between adjacent plots. Figure 19-3 illustrates a two-row plot in which the spacing between plots is wide enough to minimize interplot competition and the spacing within the plot is reduced to minimize the land area required for each plot.

Some breeders plant one cultivar as a common border between one- or two-row plots. In barley, a lodging-resistant cultivar is used as a common border to prevent genotypes with lodging susceptibility from falling on genotypes in adjacent plots, thereby causing them to lodge unnaturally. The use of a common border has been evaluated as a means of eliminating intergenotypic competition between plots for seed yield and other quantitative characters. The results of the research indicate that a common border can reduce but not eliminate interplot competition (Thorne and Fehr, 1970a). The average interplot competition for seed yield among four soybean cultivars in single-row plots spaced 50 cm apart was compared with competition of the cultivars when a common border was used (Gedge et al., 1977). Interplot competition averaged 11.0 percent in single-row plots and 8.3 percent in plots with a common border.

Plot Size and Shape

The size of plots used to evaluate genotypes varies with the character being evaluated, the amount of experimental error that is considered acceptable for

measuring a character, the experimental design, and the growth characteristics of the crop. Plots vary in size from those for a single plant that is harvested by hand to those that are wide and long enough to be harvested with the same equipment used by farmers for commercial production.

Single-Plant Plots. Individual plants commonly are evaluated in segregating populations. There is no replication of the individuals, unless vegetative propagation of clones is possible. The spacing among plots varies with the crop species involved. Gardner (1961) spaced individuals 50 by 100 cm apart when selecting for yield in maize. Burton (1974) spaced plants of a population of Pensacola bahiagrass 60 by 60 cm apart when conducting recurrent phenotypic selection for forage yield. Burton and Brim (1981) used a 46 by 46 cm spacing among soybean plants for selection of oil composition in the seed.

Single-plant plots are used for the replicated evaluation of experimental lines or cultivars by the honeycomb field design (Fasoulas, 1979). The number of plants evaluated for a line is equal to the number of replications in the experiment. Fasoulas (1981) indicated that 100 single-plant plots (replications) per line would provide satisfactory results. The plots of the lines in a test are organized in a systematic manner to permit comparison of a plant of one line with adjacent plants of other lines (Fig. 19-4). The honeycomb design has not been adopted by plant breeders for replicated evaluation of lines because it requires more labor and is less amenable to mechanization than microplots or conventional row plots.

Multiple-Plant Plots. The evaluation of experimental lines or cultivars by plant breeders is usually done in plots containing two or more plants. Plot size varies from small microplots consisting of a hill or short row to a plot with one or more rows several meters in length.

Microplots. Microplots are used to minimize the amount of seed or land required to evaluate a group of lines. In an unbordered microplot, the effects of interplot competition must be considered when determining an appropriate distance among plots (Fig. 19-5). For oats, hill plots spaced about 30 by 30 cm apart have been used (Frey, 1965), while for soybeans, a spacing of about 1 by 1 m is more common (Garland and Fehr, 1981).

The number of plants in a microplot differs among crops. A planting rate of 30 seeds per hill is satisfactory in oats (Frey, 1965), while a rate of 12 seeds per hill is used for soybeans (Garland and Fehr, 1981). When short rows are used as microplots, the plant density is comparable to that of larger row plots.

There is a lack of agreement among plant breeders concerning the effectiveness of microplots for evaluation of agronomic characters, particularly seed yield. Breeders who use microplots indicate that they are useful for eliminating inferior lines during the first year of yield evaluation. Lines with acceptable performance in microplots are evaluated in conventional row plots during subsequent years of testing, to identify those that merit release as cultivars (Frey,

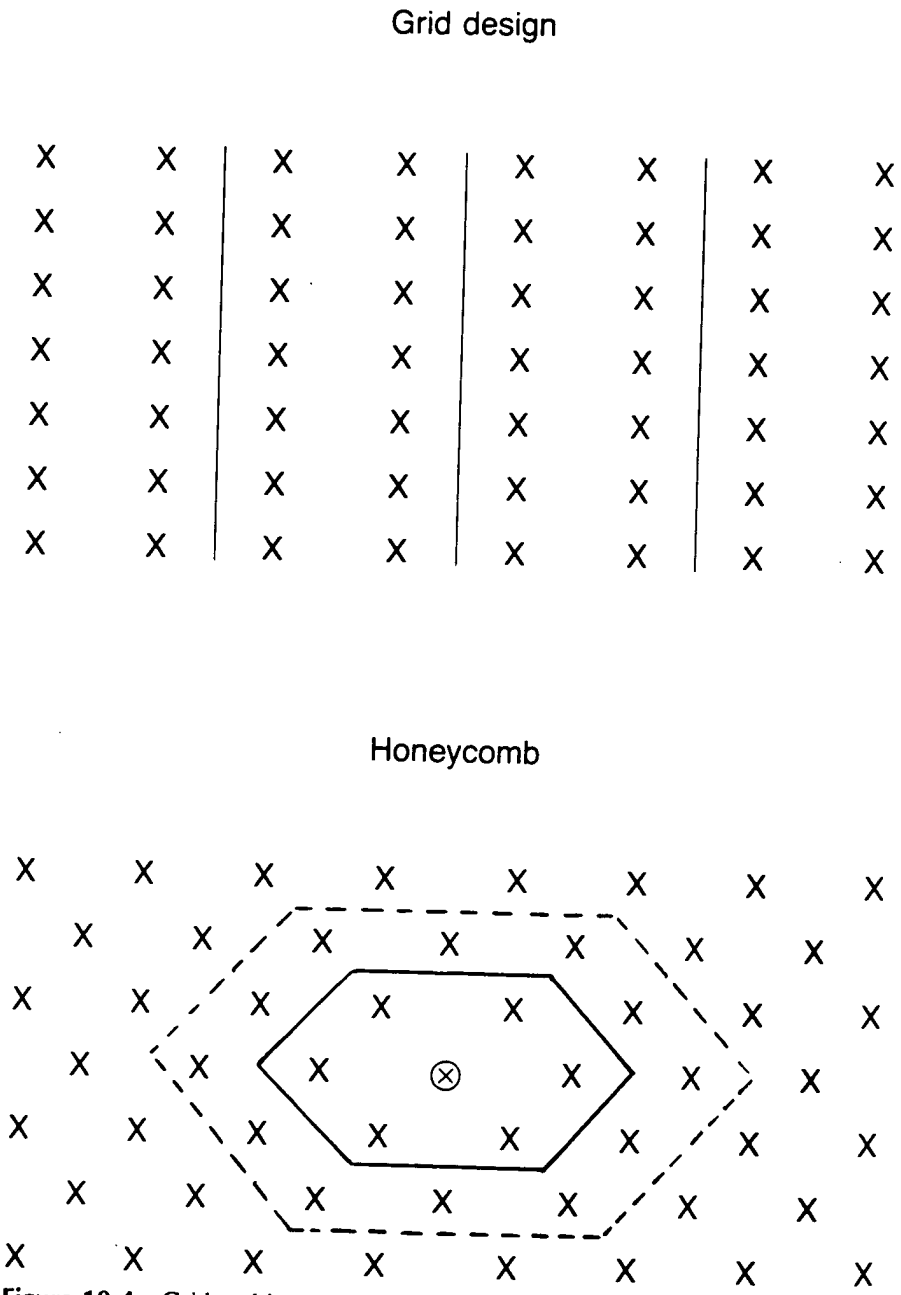


Figure 19-4 Grid and honeycomb design to select individual plants in a population. For the grid design, plants are divided into blocks and the best ones chosen from each (Gardner, 1961). For the honeycomb design, the plant at the center of the hexagon, ⊗, is compared with every other plant within the hexagon (Fasoulas, 1979). A plant is chosen only if it is superior to every other plant in the hexagon. The hexagons outlined represent two different selection intensities.

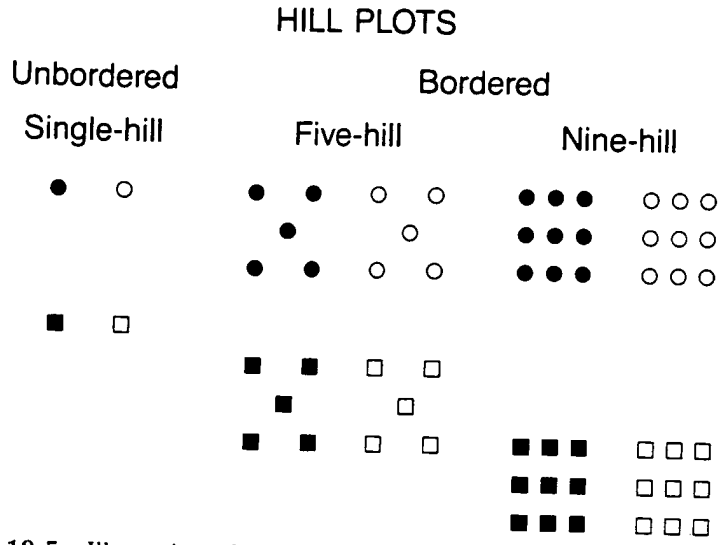


Figure 19-5 Illustration of hill plots with different cultivars designated as □, ○, ●, and ■ (Fehr, 1978).

1965; Garland and Fehr, 1981). The advantages of microplots compared with conventional row plots for the first year of yield testing are that less land is required per plot and that enough seed for replicated tests can be obtained from a single plant, which eliminates a season for seed increase. Breeders who do not use microplots are concerned about the reliability of yield data obtained from them. The coefficients of variability for microplots generally are about one and one-half to two times larger than for conventional row plots.

Row Plots. Row plots are used by virtually all plant breeders for replicated testing of genotypes. The overall plot size is determined by the number of rows, the spacing between rows, and the row length.

Single-row plots of 1 to 2 m in length are widely used for the visual evaluation of characters. Many breeders evaluate lines on the basis of their appearance in small unreplicated plots, and advance the desirable ones to replicated tests the following season. Visual selection and seed increase commonly are accomplished with the same plot.

A plot used to evaluate the yield of lines for the first time often is smaller than that employed for advanced stages of evaluation. For advanced yield tests, the breeder attempts to use a plot size that approaches or equals the dimensions considered optimal for the crop species involved. Optimum plot size is the minimum land area required to measure a character with an acceptable level of experimental error.

Optimum plot size can be determined by the use of data from a uniformity trial (Cochran, 1937). A single cultivar is planted as a solid stand, without alleys,

in an area representative of that used for yield evaluation. The cultural practices used to produce the crop are the same as those used for yield trials. The area is subdivided into small units, and the seeds or plants from each unit are harvested and weighed separately. Experimental error associated with plots of different size can be determined by making various combinations of the small units.

Optimum plot size also is determined through practical experience. The breeder often will experiment with plots of different size to find the smallest one that has an acceptable level of experimental error. Breeders often do not agree on what they consider acceptable experimental error; consequently, an optimum size for one person may not be optimum for another.

Plot width generally is determined by considerations other than the relationship of shape to experimental error. The primary factors are the number of rows required to minimize or avoid interplot competition and the width of the planting and harvesting equipment that is available. Plot width influences the percentage of land area that must be devoted to alleys between plots. Long, narrow plots require a lower percentage of alley space than do wide, short plots. This advantage is offset in bordered plots because the percentage of land area devoted to border rows decreases as the number of rows per plot increases.

Plot length provides flexibility for plot size. Before calculators and computers became readily available, row length in the United States was varied to obtain a plot size that was a fraction of an acre (one-tenth, one-twentieth, etc.) to simplify the conversion of plot yields to yields per acre. With use of computers for data summarization and analysis, this is no longer necessary.

Data Collection

The experimental error associated with the evaluation of a character is influenced by measurement errors during data collection. For characters evaluated visually, experimental error occurs whenever the data collector fails to give an identical rating to plots with an identical appearance. Reliability of the evaluation can be established readily by rating a series of plots at different times and comparing the ratings. It is essentially impossible to give visual ratings without error; therefore, the breeder must decide when the error is acceptable and when it is so large that genetic differences will be masked.

Some characters can only be evaluated efficiently with the use of an appropriate machine or instrument. Experimental error can occur because of failure to prepare a plot properly for measurement, of not obtaining a representative sample of the plot for evaluation, of using nonuniform procedures for sample preparation, and of failure of the machine or instrument to operate properly.

Preparation of a plot for data collection may begin before planting. For experimental error to be reduced, the seeds or plants of every genotype used for planting must be treated equally. If seeds or plants of genotypes to be compared

do not come from a common environment, environmental error may result. Lint yield and seedling vigor of a cotton cultivar were found to differ in plots grown from seeds obtained from different locations (Peacock and Hawkins, 1970). Seed source also has been shown to influence seed yield of soybeans (Fehr and Probst, 1971.)

In some crop species, uniformity of plant density among plots can be important in minimizing experimental error. With maize, it is a common practice to thin yield test plots to a uniform stand soon after seedling emergence. Thinning is not considered necessary with some crop species, particularly those that have the ability to branch or tiller in response to low plant density, such as barley and wheat. It also is a common practice with crops such as maize to record the number of plants per plot immediately before harvest. The yield of the plots is adjusted for plant density by an analysis of covariance, to minimize experimental error in the comparison of genotypes.

When a blank alley is used at the end of row plots, the end plants generally are more productive than those growing in the center of the plot. When end plants are harvested, yield of the plot is inflated in comparison to the yield obtained from plants growing in the center of the plot. This inflation will prevent a direct comparison of plot yields with those expected in a normal commercial planting, unless an appropriate adjustment is made for all plots. The adjustment may be made by considering the alley as part of the plot area; therefore, plot length is the distance from the center of one alley to the center of the next, instead of the distance between plants at opposite ends of a row. For example, if the length of row containing plants is 5 m and the alley is 1 m wide, the plot length for computing plot area is considered to be 6 m.

The yield inflation by end plants in a plot does not contribute to experimental error unless genotypes in a test do not respond similarly to the space in the alley. The experimental error associated with differential response of genotypes to an alley can be minimized by adjusting yields according to characteristics of the genotypes that influence this response. The end plants of soybean genotypes with late maturity give a greater yield inflation than do genotypes of early maturity. Values have been developed with which to adjust plot yields for maturity of soybean genotypes (Wilcox, 1970). More commonly, comparisons among soybean genotypes are restricted to those of similar maturity, unless plots are end-trimmed before harvest.

The only way to eliminate yield inflation by end plants is to remove the plants before harvest. This procedure, referred to as end-trimming, is a standard procedure with some crops. The end plants are removed late enough in plant development that the remaining plants in the plot cannot take advantage of the extra space. The length of row removed from each end of the plot must be long enough to include all plants that have benefited from the space provided by the alley. In soybean, 0.6 m is removed from each end of the plot (Wilcox, 1970).

The problem of a blank alley is minimized in some crops by planting the

alley with rows of a single genotype perpendicular to the test plots. The result is that the plants at the end of a plot must compete with plants in the alley, and thus their yield may not be inflated as much as is the case with a blank alley. Plants in the alley are removed immediately before the plots are harvested.

EXPERIMENTAL DESIGNS

The arrangement of genotypes in a field experiment is referred to as the experimental design. Some of the designs utilized to compare genotypes are common to research in many disciplines. Others have been developed to deal with the problem of comparing a large number of genotypes as inexpensively as possible. The experimental designs used for the initial evaluation of a large number of genotypes often differ from those used in the advanced stages of testing a few select genotypes. Alternative designs will be considered here for comparison of single plants, unreplicated genotypes in multiple-plant plots, and replicated genotypes.

Single-Plant Selection

The first evaluation step in the development of a cultivar generally is the selection of individual plants from a population. Individual plant selection also is employed in population improvement by recurrent phenotypic selection.

When single-plant selection in a population is for characters with a high heritability, the plants generally are grown in a random order and those with desirable characteristics are selected. Cultivars may be grown in adjacent plots to serve as standards with which to evaluate single plants. Date of flowering, plant height, time of maturity, and certain types of pest resistance are examples of characters for which single plants are selected without any predetermined arrangement of the individuals. They represent characteristics that are not strongly influenced by environmental variation.

Single-plant selection in a population grown in a relatively large land area can be hampered seriously by soil heterogeneity for characters with a low heritability, such as seed or plant yield. Figure 19-1 illustrates variation in soil productivity in an area where a population of plants may be grown. If plants with the highest yield are selected regardless of their location in the field, those in the area of above-average productivity will be favored. A plant with outstanding genetic potential that is located in the area with below-average productivity may be discarded. Two experimental designs are available that minimize the effect of soil heterogeneity by comparing plants that are most adjacent to each other.

Grid Design. Gardner (1961) proposed that the land area on which a population of individual plants is grown can be subdivided into blocks or grids of a limited

area (Fig. 19-4). Plants within each block are compared with each other, and the superior ones are selected. Comparisons are not made between plants from different blocks. This experimental design has been well accepted by plant breeders, particularly those conducting recurrent phenotypic selection for yield or other characters with a low heritability.

Honeycomb Design. Fasoulas (1973) developed a honeycomb design for selecting individual plants in a population (Fig. 19-4). Five aspects of the design and its implementation are unique. (a) Seeds or clones are spaced equidistantly from each other in a hexagon pattern. The name of the design was chosen because the hexagon patterns resemble a honeycomb of bees. (b) Plants are spaced far enough apart that they do not compete with adjacent individuals. At the appropriate spacing for a species, a missing plant does not influence the performance of adjacent individuals, because each plant already has sufficient space in which to develop to its full potential. (c) Homogeneous check cultivars can be included for comparison, if desired. Every plant of the check is compared with a different group of plants in the population. (d) The size of the hexagon used to select single plants determines the selection intensity in the population. The effect of soil heterogeneity is minimized because only those plants within the area of the hexagon are compared. (e) Every plant in the population is evaluated by placing it in the center of the hexagon. A plant is chosen only if it is superior to every other plant in the hexagon. By moving the hexagon, every plant is compared with a different group of plants in the population.

Comparison of the Grid and Honeycomb Designs. Both the grid and honeycomb designs reduce the problem of soil heterogeneity in the selection of characters of low heritability. In a comparison of the designs, the advantages of one are the disadvantages of the other, and vice versa.

There are three primary advantages of the grid design.

1. The spacing of plants does not have to be in a precise pattern. This facilitates the use of conventional plot equipment for planting and cultivation. Mechanized planting of the honeycomb design would require specialized equipment.
2. Selection intensity can be varied by altering the number of plants in a block and the number of plants selected. Only certain selection intensities are possible with the honeycomb design.
3. Use of a defined area for each block facilitates visual comparison of plants for selection. It is possible to compare plants within a block visually and collect data only from those with the best potential. Use of the moving hexagon for the honeycomb design makes it impractical to compare each plant with appropriate ones in its hexagon; therefore, data must be recorded for every plant, except those that are obviously inferior.

The honeycomb design has two advantages compared with the grid design.

1. Homogeneous check cultivars can be included to permit comparisons of individual plants with a standard. When one-seventh of the plants are a check, they can be arranged so that every plant in the population can be compared with a check plant. To provide adjacent plants of one check cultivar in a grid system, one-third of the area would have to be devoted to the check.
2. More than two check cultivars can be included readily in hexagons of 19 or more plants. Use of two or more check cultivars in the grid system would require that a large fraction of each block be devoted to check plants.

Unreplicated Evaluation with Multiple-Plant Plots

Plant breeders routinely conduct visual selection among lines in unreplicated plots for maturity, disease resistance, standability, and other characters of high heritability. Evaluation for yield in a single replication has been used to a limited extent to eliminate inferior lines before initiation of expensive replicated tests. With a single replication, each line is compared once with check cultivars or other lines to determine its genetic potential. A number of different arrangements are available for estimating the genetic potential of lines. One method is to compare each line with a common check cultivar (Baker and McKenzie, 1967). Figure 19-6 represents a hypothetical example of the yield of six lines in a single replication. In the figure, the yield of each line is expressed as a percentage of the yield of the check cultivar immediately adjacent to it.

Another alternative is to express the yield of each line as a percentage of the weighted average of the adjacent check plot and of the check plot two plots removed. The purpose for using a weighted average is to minimize the potential problem caused by an unusually poor yield of a check plot. In Fig. 19-6, the check cultivar adjacent to lines B and C has a much lower yield than other check cultivars. This results in an extremely high percentage for lines A and B. The weighted average of check cultivars could be computed as

$$(\tfrac{2}{3} \times \text{yield of adjacent check}) + (\tfrac{1}{3} \times \text{yield of check two plots removed}) = \text{weighted average of check cultivars}$$

The percentage yield of each line is computed as

$$\text{Line A} = \frac{59}{(\frac{2}{3} \times 55) + (\frac{1}{3} \times 39)} \times 100 = 119$$

$$\text{Line B} = \frac{70}{(\frac{2}{3} \times 39) + (\frac{1}{3} \times 55)} \times 100 = 158$$

$$\text{Line C} = \frac{53}{(\frac{2}{3} \times 39) + (\frac{1}{3} \times 48)} \times 100 = 126$$

$$\text{Line D} = \frac{51}{(\frac{2}{3} \times 48) + (\frac{1}{3} \times 39)} \times 100 = 113$$

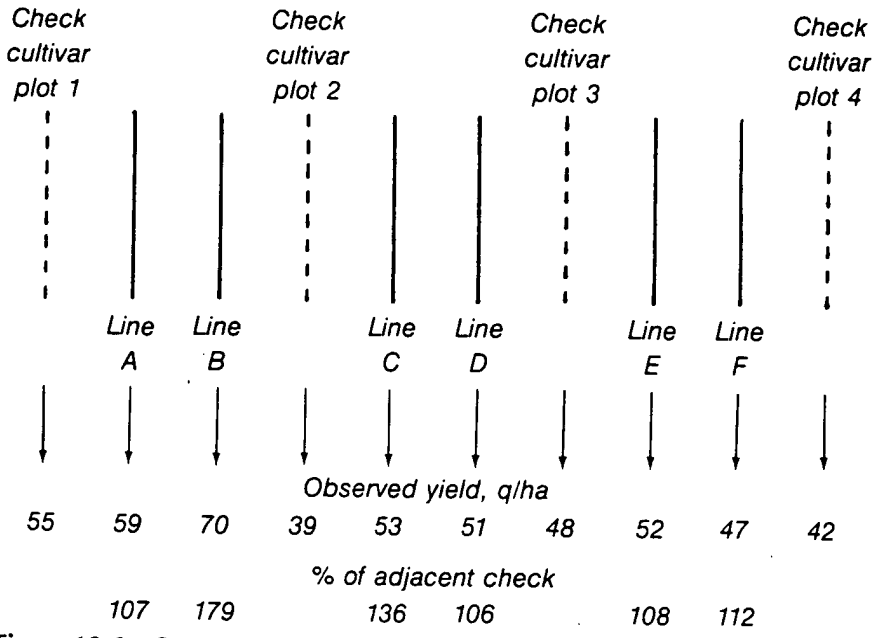


Figure 19-6 One possible arrangement of lines in a single-replication test. The performance of each line is computed as a percentage of the performance of the common check cultivar adjacent to it. Line B would be considered the superior one.

$$\text{Line E} = \frac{52}{(\frac{2}{3} \times 48) + (\frac{1}{3} \times 42)} \times 100 = 113$$

$$\text{Line F} = \frac{47}{(\frac{2}{3} \times 42) + (\frac{1}{3} \times 48)} \times 100 = 107$$

Another method used to compare genotypes in single replications is the moving mean (Mak et al., 1978; Townley-Smith and Hurd, 1973). Each genotype is compared with adjacent test genotypes, not with a check cultivar.

The disadvantage of single-replication tests is that the breeder has only one plot value with which to assess the genetic potential of a line. If by chance a line is placed on a plot of soil with above-average productivity, relative to that of plots with which the line is compared, it will seem to be genetically superior, even though it may not be. In replicated tests, the breeder will have more than one plot with which to evaluate each line. For this reason, single replications are not commonly used for yield evaluation.

Replicated Tests

Two or more independent comparisons of lines in a test provide a means of estimating whether variation in performance among lines is due to differences in genetic potential or to environmental variation. Each comparison is as rep-

lication. Replication can be accomplished by growing two or more plots of each line at one or more locations or one plot at each of two or more locations or years.

Randomization. One important consideration in the arrangement of genotypes within each replication is the degree of randomization. From a statistical viewpoint, randomization of entries is required to obtain a valid estimate of experimental error. To fulfill the requirement, each entry must have an equal chance of being assigned to any plot in a replication and an independent randomization is required for each replication.

Plant breeders understand the importance of randomization and consider it the ideal procedure for comparison of genotypes. They know that any experiment designed to estimate components of variance must be randomized. There are circumstances, however, in which plant breeders do not use complete randomization for the comparison of genotypes. Genotypes with similar characteristics may be planted next to each other to reduce interplot competition in unbordered plots. A nonrandom arrangement of genotypes among replications may be used to facilitate selection of genotypes before harvest.

Nonrandom Arrangements of Genotypes. Any discussion of nonrandom arrangements of genotypes can be misinterpreted because it may imply that randomization is not an important principle. To avoid such misinterpretation, it should be stated again that nonrandomization should only be considered when resources are not adequate to make randomization feasible. The discussion of nonrandom arrangements will include the reasons for their use, their disadvantages, and the ways procedures can be modified to permit effective randomization.

Nonrandomization Among Replications. It is common to delay replicated tests for yield until genotypes have been visually selected in unreplicated plots for characteristics such as lodging, height, and maturity. To reduce the length of time for cultivar development, the season for evaluation in unreplicated plots can be eliminated by growing genotypes in replicated plots, visually selecting those with desirable characteristics, and harvesting only the plots of selected genotypes for yield evaluation (Garland and Fehr, 1981). When visual selection is based on the performance of genotypes in all of the replications, it is necessary to evaluate each plot, summarize the data, make the selections, and identify the plots of selected genotypes that should be harvested. The length of time between plot evaluation and harvest may be only a few days when characteristics of interest are not expressed until plant maturity. If several thousand genotypes are randomized in two or more replications, summarization of data and identification of plots to be harvested can be difficult or impossible to accomplish in only a few days. The use of the same arrangement of genotypes in each replication makes the job practical.

When genotypes are in the same position within each replication, the data for plots of each genotype are recorded in adjacent columns (Fig. 19-7). Sum-

Nonrandom

Plot	Entry	Replication 1	2	3
1	1			
2	2			
3	3			
4	4			
5	5			
6	6			

Random

Plot	Entry	Replication 1
1	4	
2	1	
3	6	
4	3	
5	5	
6	2	

Plot	Entry	Replication 2
1	5	
2	4	
3	2	
4	1	
5	6	
6	3	

Plot	Entry	Replication 3
1	2	
2	6	
3	3	
4	5	
5	1	
6	4	

Figure 19-7 Field book pages for recording the data of genotypes grown in three replications. Nonrandom arrangement of genotypes involves one page, whereas a random arrangement involves three separate sections on one or more pages.

marization of data is complete as soon as the last plot is rated. Genotypes with undesirable characteristics in one or more replications can be identified and discarded. The plots of desirable genotypes are readily identified for harvest because they are in the same position in each replication.

The disadvantages of nonrandomization relate to the fact that the same genotypes are always adjacent to each other, which can have negative effects on the comparison of genotypes.

1. In unbordered plots, intergenotypic competition can bias the performance of genotypes more seriously in a nonrandom than in a random arrangement. When a poor competitor is bordered by a good competitor, yield of the poor competitor can be reduced and that of the good competitor increased in every replication. There is no opportunity for a genotype to occur next to others with a more similar competitive ability.
2. In unbordered plots, a genotype that dies or is unusually weak in all replications can prevent the accurate evaluation of adjacent genotypes. The performance of adjacent genotypes would never be tested in replications where they were next to healthy genotypes.
3. No unbiased estimate of experimental error can be obtained.

The need to use nonrandomization of genotypes among replications can be avoided by improving the efficiency of procedures for data summarization and evaluation. An efficient procedure would include the use of a computer. Data would have to be entered rapidly into the computer, possibly by entering plot data into an electronic recorder in the field and electronically transferring the information to the computer. Computer programs would be needed to summarize the data and make selections on the basis of standards established by the breeder. Plot identification information for selected genotypes would have to be provided for harvest.

Grouping Similar Genotypes Within Replications. The evaluation of genotypes in unbordered plots can be hampered by bias from intergenotypic competition. Plant characteristics that often contribute to intergenotypic competition in a crop include such factors as differences in height and time of maturity. To reduce intergenotypic competition, genotypes with similar characteristics may be grouped within replications. The position of each genotype may be varied from one replication to the next. This procedure, sometimes referred to as restricted randomization, has the advantage of reducing the effects of intergenotypic competition in unbordered plots. The primary disadvantage is that all genotypes in a test cannot be compared with the same level of confidence. Genotypes within a group are spaced closer to each other than genotypes in different groups and are less affected by environmental variation among plots.

The use of bordered plots eliminates the need for grouping genotypes. The performance of genotypes in plots is not influenced by intergenotypic compe-

tition; therefore, randomization is practical. An increase in land, seed, and other resources will be needed for replacement of unbordered plots with bordered ones.

Experimental Designs for Replicated Tests. The arrangement of genotypes in replicated tests involves primarily the use of either the randomized complete-block design or incomplete-block designs. The Latin square is used only in special circumstances when the number of entries is small (Cochran and Cox, 1957). The honeycomb design can be used for replicated testing but is considered too difficult to implement for a large number of lines (Fasoulas, 1981).

The differences between the randomized complete-block and incomplete-block designs relate to their ability to account for environmental variation within a replication. The two types of design differ in restrictions on the size of a replication, randomization procedures, analysis of data, and comparisons among genotypes.

The terms complete-block and incomplete-block refer to the arrangement of genotypes in an experiment (Fig. 19-8). A block and a replication are equivalent in a randomized complete-block design. A block contains all of the genotypes in the test and is considered complete. Genotypes are divided into more than one block within each replication of an incomplete-block design. The blocks are considered incomplete because they contain only part of the genotypes. A number of different types of incomplete-block designs are available (Cochran and Cox, 1957). The most common types used in plant breeding are referred to as lattices. In a lattice design, a replication is divided into blocks that collectively contain all the genotypes in a test (Fig. 19-8).

The incomplete-block designs are intended to provide more control over environmental variation within a replication than is possible with the complete-block design. The ideal situation for genotype evaluation would be to test each genotype in the same plot, thus avoiding any environmental variation caused by differences in soil fertility, moisture, and other factors within a field. This is not possible, so the next best approach is to adjust the performance of each genotype according to the relative productivity of the plot in which it is evaluated. If one plot has better fertility and moisture than the average for all plots in a replication, the performance of a genotype in that plot will be adjusted downward. A genotype in a plot with lower productivity than the average will have its performance adjusted upward.

Although individual plot adjustments are not possible, the lattice designs permit the performance of a genotype to be adjusted upward or downward according to the productivity of the blocks in which it was grown. The randomized complete-block design does not divide the replication into smaller units and is not able to adjust the performance of a genotype for environmental variation within replications.

The effectiveness of the lattice design in accounting for environmental variation within replications depends on the pattern of variation. Figure 19-9 shows two replications with variation in soil productivity. The soil productivity in

Block						
	Replication 1					
1	1	2	3	4	5	6
2	7	8	9	10	11	12
3	13	14	15	16	17	18
4	19	20	21	22	23	24
5	25	26	27	28	29	30
6	31	32	33	34	35	36
7	37	38	39	40	41	42
	Replication 2					
1	7	13	19	25	31	37
2	1	14	20	26	32	38
3	2	8	21	27	33	39
4	3	9	15	28	34	40
5	4	10	16	22	35	41
6	5	11	17	23	29	42
7	6	12	18	24	30	36
	Replication 3					
1	12	17	22	28	33	38
2	2	13	24	29	35	40
3	4	9	20	25	36	42
4	6	11	16	27	32	37
5	1	7	18	23	34	39
6	3	8	14	19	30	41
7	5	10	15	21	26	31

Figure 19-8 Lattice design for an experiment with 42 entries and three replications. (Adapted from Cochran and Cox, 1957.) For a randomized complete-block design, there are no blocks within a replication and the entries are assigned at random to the 42 plots.

replication 1 increases from left to right. The blocks of the lattice design are arranged in a pattern that effectively measures the variation, as evidenced by differences in the mean for each block. The variation in soil productivity in replication 2 does not fit a consistent pattern. Much of the variation occurs within blocks, and the mean performance of the blocks is relatively similar. The lattice

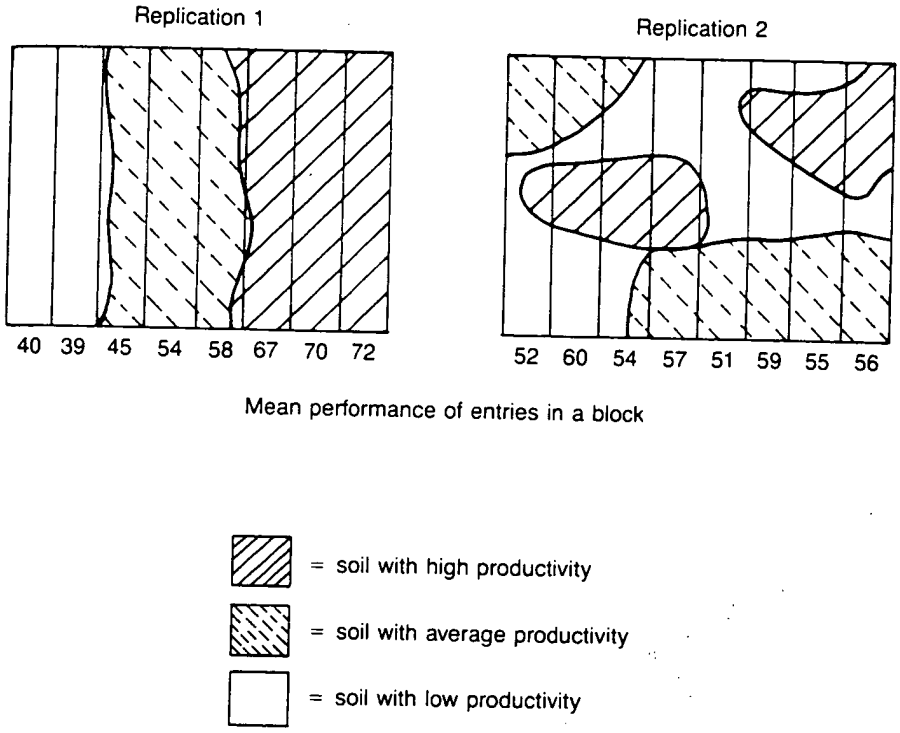


Figure 19-9 The effect of the pattern of variation in soil productivity on the effectiveness of the lattice design in accounting for environmental variation within a replication. The lattice would be more effective in replication 1 than in replication 2.

design cannot adjust for differences in productivity within a block; therefore, it would not be as effective in replication 2 as in replication 1.

The effectiveness of the lattice design compared with the randomized complete-block is expressed as relative efficiency. Relative efficiency is computed as a ratio of mean squares for experimental error of the two types of design.

$$\text{Relative efficiency} = \frac{\text{mean square for error of lattice}}{\text{mean square for error of randomized complete-block}} \times 100$$

The ratio is used to determine the number of replications that would have to be used with the randomized complete block to achieve a precision in detecting differences among the means of genotypes equal to that with a lattice design. A relative efficiency of 150 percent indicates that 50 percent more replication would have been needed with a randomized complete-block design than with a lattice.

The two types of design differ in the flexibility that is possible in a test. The randomized complete-block can accommodate any number of genotypes or replications. The lattice design requires that a specified number of genotypes and replications be included. For example, no lattice design can be used with 44, 58, or 74 genotypes. There is no restriction in a randomized complete-block for the length and width of a replication. For example, a test with 72 entries could be planted 8 plots long by 9 plots wide or 6 plots long by 12 plots wide. The shape of replication for a particular number of genotypes in a lattice is not as flexible. A test with 72 entries could be planted 8 plots long by 9 plots wide, not 6 plots long by 12 plots wide.

The randomization of an experiment and statistical analysis of data are more complex for a lattice than for a randomized complete-block. This can be important if the work is done by hand, but not if done by computer. Computer programs are available that will readily accommodate either type of design.

EQUIPMENT FOR EFFICIENT EVALUATION OF GENOTYPES

The efficient evaluation of a large number of genotypes is important for genetic improvement. Plant breeders have been actively involved in the development of equipment that permits them to evaluate more genotypes with equal or greater quality than was previously possible. The equipment ranges from simple hand devices to sophisticated computers.

Each crop has unique characteristics that influence the type of equipment used. Even for a certain crop, breeders differ as to the type of equipment they consider most desirable. Here only a small sample of available equipment will be used to illustrate how large numbers of genotypes are evaluated by plant breeders.

Preparation of Seed for Planting

The main steps involved in preparing a field experiment include packaging the seed and placing it in the proper arrangement for planting. Computers can be used to randomize entries and assign plot numbers. The computer system can print an adhesive label for each packet of seed to be packaged. The label contains the plot number, the entry number, and other information of value to the breeder. The plot and entry information also can be printed on pages used to record data in the field. The same work can be done by hand, but would require a large amount of labor and would be more subject to human error.

Seed is counted by hand or by electronic counting devices. If the number of seeds for a plot is large and precise numbers are not required, the seeds may be measured by volume.

Planting

Rapid planting of plots can be accomplished with engine-driven planters. Multiple-row plots may be planted from a single packet when each row does not require the exact same number of seeds. The seed is passed through a divider that separates the seed into a fraction for each row. The divider may be a powered spinning device or a gravity system.

The planter can move through the field without stopping. Seed for a row is placed in a container above a planting cone. When the row is to be planted, the container is lifted and the seed drops onto the planting cone. Two types of cones are used to distribute seed along the row. For one type, the base turns and carries the seed to the outlet. There it is knocked from the base by a stationary plate, falling through the outlet to the soil. This type of cone is used for relatively small seeds that do not roll easily, such as barley. The second type has fins mounted on the center cone. The seed falls onto a stationary base and is dragged by the fins to the outlet. The fins are well suited to relatively large seeds, particularly those that have a tendency to roll easily, such as maize and soybean. The length of a plot is a function of the distance traveled by the planter before all the seed has left the cone. At a constant ground speed, a cone must turn faster for short rows than for long rows. Adjustment of the speed of the cone rotation can be accomplished readily by several mechanical systems.

While the seed for one plot is being planted, the seed for the next plot is put in the container above the cone. There are a number of ways to determine when the container should be lifted to begin a plot. One way is to mark the beginning and end of each plot in the field before planting starts. When the planter reaches the beginning of a plot, the operator lifts the containers manually or electronically. The advantage of this procedure is that the location of each plot can be identified as soon as planting is complete. The second way is to use a cable extended across the field that has knobs spaced along it. The spacing between knobs is equal to the length of the plot and the alley. For plots that have rows 5 m long with a 1 m alley between them, the knobs would be spaced 6 m apart. As the planter passes by the cable, the knobs signal when the container should be lifted manually, or it activates an electronic tripping device. The cable is moved after each pass across the field. Use of the cable saves time at planting by eliminating the need to mark the start and end of plots manually.

Weed Control

Weed control is accomplished by the use of chemicals, cultivation, and hand weeding. The chemicals generally are those applied for weed control in commercial production of the crop. Cultivation equipment may be especially designed for use in research fields or may be the same equipment used commercially.

Preparation of Plots for Harvest

Trimming of plots to a constant length before harvest is done manually or with specialized equipment. Plots of small grains generally are trimmed to a constant length early in the season when the plants are about 30 cm tall. A rototiller or mower is passed along the end of each plot to kill the unwanted plants. The rototiller may be mounted on a tractor or may be a self-propelled unit that a person walks behind. Plots of soybean can be cut to a constant length with rotary mowers before seed filling begins. Two mowers are attached to a pipe so that they are separated by a distance equal to the desired plot length, and are driven perpendicular to the length of the rows.

Harvest

The most common type of harvester for the measurement of forage yield in the United States is a self-propelled flail chopper. The machine cuts the plants with a rotating flail that throws the cut portion into a collection point behind the driver. The plant material for a plot may be collected in a plastic container and weighed on a stationary scale set up in the field. To eliminate the labor required to use containers, an electronic scale can be mounted on the machine. The plant material is weighed and then it is discarded into a wagon.

The harvest of plots for their seeds is conducted with three different procedures or types of equipment. One procedure is to collect that part of the plant that bears the seed, weigh it directly, or carry it to a stationary machine for threshing. The plant part may be removed by hand or may be collected with a machine, such as a mower with a collection basket mounted behind the sickle. The harvested sample may be threshed immediately or dried for a period of time before threshing. One popular type of stationary machine is the Vogel thresher. The plants pass vertically through the machine as they are threshed. For a second type of stationary thresher, the material passes through the threshing cylinder and falls on a sieve that helps separate the seed from the plant debris. Air is used to separate the seed and the plant debris in both types of machine.

The second procedure for harvesting plots is to use a self-propelled thresher specifically designed for small plots. The plant part with the seed is gathered into the machine and passes through a threshing cylinder, then the seed and plant debris are separated by sieves and air. The seed may be placed into a bag and saved or may be weighed immediately and discarded. Seed harvested from self-propelled machines generally is more subject to mixtures than that harvested with a stationary thresher.

The third type of equipment is a commercial combine modified for the harvest of small plots. A commercial unit is used only when the amount of seed harvested

from a plot is relatively large and is not saved for planting. Modifications of the commercial combine include reduction of the number of rows harvested and the addition of equipment for weighing the seed.

Data Collection

Usually a number of characters are measured on each plot, such as height, standability, and yield. The data may be recorded in a field book, then manually entered into the computer for statistical analysis. Alternatively, the information may be recorded in an electronic data collector and transferred directly to the computer. This saves time and reduces the possibility of human error. Plot and entry designations also can be recorded on labels that can be read into the data collector by an electronic scanner.

Data Analysis

Computers facilitate the selection of lines by summarizing data in whatever manner is beneficial to the breeder. They save an extensive amount of time, minimize human error, and permit data to be summarized in a short period of time.

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